

5 where the cells contain any of the nucleic acids or polypeptides of claims 1-86 and where the cells are shown to cleave the β secretase site of any peptide having the following peptide structure, P2, P1, P1', P2', where P2 is K or N, where P1 is M or L, where P1' is D, where P2' is A. The method of claim 111 where P2 is K and P1 is M. The method of
 10 5 claim 112 where P2 is N and P1 is L.

Any bacterial cell comprising any nucleic acids or peptides in claims 1-86 and 92-107. A bacterial cell of claim 114 where the bacteria is *E. coli*. Any eukaryotic cell comprising any nucleic acids or polypeptides in claims 1-86 and 92-107.
 15

Any insect cell comprising any of the nucleic acids or polypeptides in claims 1-86 and 92-107. A insect cell of claim 117 where the insect is sf9, or High 5. A insect cell of claim 100 where the insect cell is High 5. A mammalian cell comprising any of the nucleic acids or polypeptides in claims 1-86 and 92-107. A mammalian cell of claim 120 where the mammalian cell is selected from the group consisting of, human, rodent, lagomorph, and primate. A mammalian cell of claim 121 where the mammalian cell is selected from the group consisting of human cell. A mammalian cell of claim 122 where the human cell is selected from the group comprising HEK293, and IMR-32. A mammalian cell of claim 121 where the cell is a primate cell. A primate cell of claim 124 where the primate cell is a COS-7 cell. A mammalian cell of claim 121 where cell is selected from a rodent cells. A rodent cell of claim 126 selected from, CHO-K1, Neuro-2A, 3T3 cells. A yeast cell of claim 115. An avian cell of claim 115.
 20

Any isoform of APP where the last two carboxy terminus amino acids of that isoform are both lysine residues. In written descrip. Define isoform is any APP polypeptide, including APP variants (including mutations), and APP fragments that exists in humans such as those described in US 5,766,846, col 7, lines 45-67, incorporated into this document by reference. The isoform of APP from claim 114, comprising the isoform known as APP695 modified so that its last two having two lysine residues as its last two carboxy terminus amino acids. The isoform of claim 130 comprising SEQ. ID. 16. The isoform variant of claim 130 comprising SEQ. ID. NO. 18, and 20. Any eukaryotic cell line, comprising nucleic acids or polypeptides of claim 130-132. Any cell line of claim 133 that is a mammalian cell line (HEK293, Neuro2a, best - plus others. A method for identifying inhibitors of an enzyme that cleaves the beta secretase cleavable site of APP comprising:
 30
 40
 45
 50

5 a) culturing cells in a culture medium under conditions in which the enzyme causes processing of APP and release of amyloid beta-peptide into the medium and causes the accumulation of CTF99 fragments of APP in cell lysates,

10 b) - exposing the cultured cells to a test compound; and specifically determining whether the test compound inhibits the function of the enzyme by measuring the amount of amyloid beta-peptide released into the medium and or the amount of CTF99 fragments of APP in cell lysates;

15 c) identifying test compounds diminishing the amount of soluble amyloid beta peptide present in the culture medium and diminution of CTF99 fragments of APP in cell lysates as Asp2 inhibitors.

20 The method of claim 135 wherein the cultured cells are a human, rodent or insect cell line. The method of claim 136 wherein the human or rodent cell line exhibits β secretase activity in which processing of APP occurs with release of amyloid beta-peptide into the culture medium and accumulation of CTF99 in cell lysates. A method as in claim 25 137 wherein the human or rodent cell line treated with the antisense oligomers directed against the enzyme that exhibits β secretase activity, reduces release of soluble amyloid beta-peptide into the culture medium and accumulation of CTF99 in cell lysates. A method for the identification of an agent that decreases the activity of a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), the method 30 comprising:

a) determining the activity of said Hu-Asp polypeptide in the presence of a test agent and in the absence of a test agent; and

35 b) comparing the activity of said Hu-Asp polypeptide determined in the presence of said test agent to the activity of said Hu-Asp polypeptide

25 determined in the absence of said test agent;

40 whereby a lower level of activity in the presence of said test agent than in the absence of said test agent indicates that said test agent has decreased the activity of said Hu-Asp polypeptide. The nucleic acids, peptides, proteins, vectors, cells and cell lines, and assays described herein.

45 30 The present invention provides isolated nucleic acid molecules comprising a polynucleotide that codes for a polypeptide selected from the group consisting of human aspartyl proteases. In particular, human aspartyl protease 1 (Hu-Asp1) and two alternative splice variants of human aspartyl protease 2 (Hu-Asp2), designated herein as Hu-Asp2(a) and 50

Hu-Asp2(b). As used herein, all references to "Hu-Asp" should be understood to refer to all of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b). In addition, as used herein, all references to "Hu-Asp2" should be understood to refer to both Hu-Asp2(a) and Hu-Asp2(b). Hu-Asp1 is expressed most abundantly in pancreas and prostate tissues, while Hu-Asp2(a) and Hu-Asp2(b) are expressed most abundantly in pancreas and brain tissues. The invention also provides isolated Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) polypeptides, as well as fragments thereof which exhibit aspartyl protease activity.

In a preferred embodiment, the nucleic acid molecules comprise a polynucleotide having a nucleotide sequence selected from the group consisting of residues 1-1554 of SEQ ID NO:1, encoding Hu-Asp1, residues 1-1503 of SEQ ID NO:3, encoding Hu-Asp2(a), and residues 1-1428 of SEQ ID NO:5, encoding Hu-Asp2(b). In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent conditions to a polynucleotide encoding Hu-Asp1, Hu-Asp2(a), Hu-Asp2(b), or fragments thereof. European patent application EP 0 848 062 discloses a polypeptide referred to as "Asp 1," that bears substantial homology to Hu-Asp1, while international application WO 98/22597 discloses a polypeptide referred to as "Asp 2," that bears substantial homology to Hu-Asp2(a).

The present invention also provides vectors comprising the isolated nucleic acid molecules of the invention, host cells into which such vectors have been introduced, and recombinant methods of obtaining a Hu-Asp1, Hu-Asp2(a), or Hu-Asp2(b) polypeptide comprising culturing the above-described host cell and isolating the relevant polypeptide.

In another aspect, the invention provides isolated Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) polypeptides, as well as fragments thereof. In a preferred embodiment, the Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) polypeptides have the amino acid sequence given in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6, respectively. The present invention also describes active forms of Hu-Asp2, methods for preparing such active forms, methods for preparing soluble forms, methods for measuring Hu-Asp2 activity, and substrates for Hu-Asp2 cleavage. The invention also describes antisense oligomers targeting the Hu-Asp1, Hu-Asp2(a) and Hu-Asp2(b) mRNA transcripts and the use of such antisense reagents to decrease such mRNA and consequently the production of the corresponding polypeptide. Isolated antibodies, both polyclonal and monoclonal, that binds specifically to any of the Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b) polypeptides of the invention are also provided.